TITLE: *In vitro* inhibition of *Escherichia coli, Salmonella* spp. and *Staphylococcus aureus* by *Lactobacillus reuteri* metabolites

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ABSTRACT:
Lactobacillus reuteri is a lactic acid bacteria able to inhibit pathogenic bacteria, its action is attributed to the combination of different mechanisms, such as the production of lactic acid and reuterin. *L. reuteri* can produce reuterin, an antimicrobial compound, in media where there is excess of glycerol under anaerobiosis. The objective of this study was to verify if *L. reuteri* strain LR 92 (Sacco DSM 26866) metabolites can inhibit the growth of *Escherichia coli* ATCC 25922*, Salmonella* spp. ATCC 13076 and *Staphylococcus aureus* ATCC 25923 *in vitro.* Two treatments were used: crude extract (EB) and concentrated extract (EC), obtained from the fermentation of the bacteria in MRS broth with 200 mM glycerol. After 24 h of fermentation, the samples were centrifuged at 6300 xg for 10 minutes at 28 °C, the supernatant (EB) was rotaevaporated at 50 °C until concentrated 10 times to obtain EC. The samples were filtered through a 0.22 µm membrane filter before the analysis. Inhibition assays were performed by well diffusion using agar BHI in triplicate per dish, with a positive control (antimicrobial gentamicin) and negative control (MRS broth). In this well diffusion agar assay each well contained 50 µL of treatment or control and results were interpreted after 24 of incubation at 37 °C. Reuterin analysis was performed according to a photometric method, pH measurements were made with a digital pH meter and acidity measurements were made by titration with a 0.1 M NaOH solution. In the well diffusion agar essay, the EB treatment showed no inhibition halos against the pathogenic bacteria. In contrast, the EC treatment results showed inhibition zone diameters of 18.7 ± 0.6 mm, 21.0 ± 1.0 mm and 19.3 ± 1.2 mm for *E. coli*, *Salmonella* spp. and *S. aureus*, respectively. The quantification of reuterin was 0.23 ± 0.03 mM and 2.76 ± 0.30 mM for EB and EC, respectively. The titratable acidity was 2.00 ± 0.10% for EB and 11.45 ± 0.21% for EC. The pH values were 4.16 ± 0.10 and 4.51 ± 0.09 for EB and EC, respectively. The results obtained suggest that the inhibitory activity observed was due to the concentration of lactic acid, the low pH of the extracts and the production of reuterin. However, this inhibition was only favorable for EC, showing that *L. reuteri* metabolites can have a potential antimicrobial activity when concentrated, in the tested conditions.

Keywords: lactic acid bacteria, reuterin, well diffusion agar assay.

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